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Bionanotechnology with Membrane Proteins: Mechanics and Electronics

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1. Introduction

In recent years there has been an upsurge of research in nanotechnology fuelled by interest in miniaturising electronic/photonic devices and robotics, developing tools for biological research, medical and pharmacological applications, etc. Nanotechnology is perfectly realised in biological systems; cells replicate, communicate, sense the environment, move, feed and die using nanoscale proteins and nucleic acids. Therefore, the miniaturisation of devices naturally points to biological evolved nanomachinery to find materials and assembly strategies at the nm scale. There are many hurdles to be overcome so that fully functional systems may be generated. Technology will emerge solely after a good scientific knowledge of the systems has been achieved. Understanding the functioning of biomachines, the development of a complete set of different biomolecular components and the ability to interface or assemble them, are some of the challenges to be faced in the near future. The problems involved in controlling and coordinating several biomolecular machines will come next.

Particularly interesting in the quest for bionanotechnology are membrane proteins. These compact, highly versatile proteins work inserted in the lipid bilayers that separate the cell from its surroundings. They can act as transporters and pores, motors, switches and pumps of ions and analytes. They can sense touch, temperature, light and volume, and transduce energy. The large variety of functions of membrane proteins promises an equally large number of possible applications and devices. Membrane proteins constitute about 30% of all the proteins encoded in the human genome, and represent the most important class of drug targets: about 50%. However, only about 2% of the 3D structures in the Protein Data Bank are membrane proteins. The number of high-resolution structures remains even smaller, largely because of the difficulties in crystallizing them. Recently, 3D structures of rhodopsins, water, potassium and chloride channels and light harvesting complexes have been resolved, and with them the details of the atomic design that evolution has used to create the selectivity, gating and conduction mechanisms of these bionanomachines are starting to be revealed.

Our aim is to use membrane proteins for constructing nanodevices. For achieving this objective we focus on the biophysical study of structure, the forces behind protein-protein and protein-lipid interactions and function. Additionally, we search for artificial environments that allow protein function while enabling a macroscopic read-out.

Among other systems, we have concentrated our work on the study of synthetic peptides and bacteriorhodopsin (bR), a robust membrane protein that is found in nature forming "purple membrane" crystals in the plasma membrane of halophilic bacteria (Halobacterium Salinarum). bR is a light-driven proton pump, that converts light energy into an electrochemical gradient across the bacterial plasma membrane. The proton gradient is used to synthesize ATP by ATPases. A broad range of applications have been proposed for which this protein shows comparative advantage. These include random access thin film memories, neural-type logic gates, photon counters and photovoltaic converters, reversible holographic media, artificial retinas, picosecond photodetectors, spatial light modulators, as multilevel logic gates, optical computing, etc.

2. Results

In our work we use Atomic Force Microscopy (AFM) and Dynamic Force Spectroscopy (DFS) to study the structure and the main mechanisms that membrane proteins use for functioning inserted in lipid bilayers. In particular the role of tryptophan (TRP) residues in anchoring has been studied by DFS of synthetic WALP peptides inserted in gel phase lipid bilayers of different thickness. Molecular Dynamics simulations were used to give an atomic detail interpretation of the experiments [1]. The results are useful for understanding of role of TRP residues in the function of membrane proteins such as bR.

AFM and DFS have been used to study the mechanical properties of purple membrane. The effect of salt and pH in the interaction between lipids and proteins, the electrostatics and the membrane cohesion have been investigated [2].The results highlight the leaflet asymmetry , both in composition and structure necessary for pumping protons across the membrane.

Purple membranes have been wrapped around metallic N-doped and Fe-doped carbon nanotubes. Different chemical treatments of the nanotubes producing hydrophilic as well as hydrophobic surfaces have been used in order to achieve the best coverage keeping protein activity. I vs. V measurements have been conducted in order to characterise the hybrid structures [3].

Tunnelling and conductive AFM have been used to map the electrostatics and capacitance of purple membranes adsorbed on metallic substrates such as gold and graphite. This information is used to interpret electrical I vs. V measurements of purple membrane adsorbed on 10 nm gap electrodes [4].

Finally, research towards the monomerisation of bR and subsequent trapping in nanogap electrodes for the realisation of single protein devices will be presented.

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